<table>
<tr><td>PMRA No.: 2364887 (hyperlink)</td><td>Study title reference

Semi-field test: effects of oil-seed rape grown from seeds dressed with A 9700 B on the honey bee (Apis mellifera L.)
Author: Nengel, S.
Report Date: 10-NOV-98
EFSA study reference: 98081/01-BZEU</td></tr>
<tr><td>MRID No.:</td><td>No</td></tr>
<tr><td>GLP:</td><td>Yes.</td></tr>
<tr><td>Type of Study:</td><td>Semi-field study with honeybees oil seed rape treated with A9700B (thiamethoxam). A9700B is Cruiser 350 FS, which is a relevant EUP for Canada, registered for Wheat, barley, corn, rye, triticale, buckwheat, millet, sorghum, soybeans and beans at 62.5 g ai/ha maximum rate, or 0.25 mg per kernel for corn (up to 100 g ai/100 kg seed). The relevancy of the rate and crop will also be considered.

The colonies were kept in the tunnels for 9 days, after which time they were moved to cages.
<table>
<tr><th>Activity</th><th>DAE</th><th>Date</th></tr>
<tr><td>Brood control before the bees were set up into the cages</td><td>-1</td><td>03AUG1998</td></tr>
<tr><td>Set up of test hives</td><td>-1</td><td>03AUG1998</td></tr>
<tr><td>1st evaluation of mortality and flight intensity</td><td>1</td><td>04AUG1998</td></tr>
<tr><td>Last evaluation of mortality and flight intensity</td><td>8</td><td>11AUG1998</td></tr>
<tr><td>1st brood control after the bees were set up into the cages</td><td>9</td><td>12AUG1998</td></tr>
<tr><td>2nd brood control after the bees were set up into the cages</td><td>28</td><td>31AUG1998</td></tr>
</table>
Remark: DAE = days after exposure</td></tr>
<tr><td>End-use product tested and rate of application</td><td>A9700B (thiamethoxam) (at 1200 mL/100 kg seed)</td></tr>
<tr><td>Crop</td><td>Seed treated oil seed rape (Brassica napus)</td></tr>
<tr><td>Plot size</td><td>The size of each plot covered with Brassica napus was approximately 3.6 m by 2.4 m. Details about sowing are given in Tab. 1.
The dimensions of the floor of the test cages were 4.8 m x 3.6 m and the height was 2 m. The cage frames were covered with light plastic gauze. The test cages were placed over the plots before the first flowers were open.</td></tr>
<tr><td>Drilling of seeds</td><td>Equipment used for drilling treated seed was calibrated prior to use. The seeding machine was a pneumatic seeder (Accord Pneumatic I A). Details of drilling machine is in Appendix I.</td></tr>
<tr><td>Number of replicates</td><td>Three (3 cages with one colony each).</td></tr>
<tr><td>Guideline:</td><td>This GLP compliant study was conducted in compliance with the European Council Directive 91/414/EEC (1997), IVA (BEUTEL et al. 1992) and OEPP/EPPO Guideline No. 170 (3) (2001).</td></tr>
<tr><td>Deviations:</td><td>The condition of the colonies and development of bee brood was checked 9 days and</td></tr>
</table>

	29 days after bees were set up into the cages. This deviation did not affect the validity of the study.
Study Design:	**The effects of spring oil-seed rape dressed with A-9700 B were tested on the honey bee (*Apis mellifera* L.) under semi-field conditions. Plots with A-9700 B treated oil-seed rape (*Brassica napus*) were used as test substance variant. Plots with untreated oil-seed rape served as control. The effect of the test substance was examined on small bee colonies in cages placed over the plots prior to the full flowering of the *Brassica napus*. Observations of mortality, foraging activity and behaviour of the bees were performed 1 day (DAE 1) and up to 8 days (DAE 8) after the bees were set up into the cages. The conditions of the colonies and the brood development were checked one day before the first evaluation, 9 days (DAE 9) as well as 28 days (DAE 28) after the bees were set up into the cages.**
Measurements:	Mortality: Assessed at edge of area covered with flowering plants and in the bee trap at the entrance of the colonies. Flight activity: Measured 3 times during bee flight activity on day 1, 2 and 3 and then once per day from day 4-8. Observations were about 5 minutes per tent. The number of bees that were foraging on flowers and flying over the crop were counted on a square of 1 metre squared. Avoidance was also assessed. Condition of the colonies, development of bee brood: Assessed one day before first evaluation as well as 9 days and 28 days after the bees were set up into the cages. The following were assessed: • Strength of the colony (number of combs covered with bees) • Presence of a healthy queen (presence of eggs, presence of queen cells) • Estimate of the pollen storage area and area with nectar • Estimate of the area containing eggs, larvae and capped cells The amount of eggs, larvae and capped brood were given in percent of total brood population for each type of brood.
Collection of residues? Results as presented by study author.	**No.**
Conclusions (study author) (EAD changes in redink)	**Mortality:** The mortality in the test substance variant was in the same range as mortality of the control variant. The average mortality during the evaluation days day 1to 8 was 14.1 dead bees/colony and day in the treated variant and 17.8 dead bees/colony and day in the control variant. Based on mortality on individual days of observation, there was slightly higher mortality on the first day after introduction in the treatment group (17.7 dead bees) compared to the control (10 dead bees). Thereafter, the control mortality and treatment mortality showed a similar trend. A higher number of dead bees were found in the edge of crop assessment (compared to the bee trap assessment) in the last few days of the experiment, in both the treatment and control groups. However, it should be noted that there was a low number of dead bees in

	either group. **Foraging activity:** Regarding the flight intensity only negligible differences were observed between the test substance and control variant. During the entire observation period the average flight intensity was 7.3 bees/metre squared in the three replications of the test substance variant compared to 7.6 bees/metre squared in the control variant. **Brood development:** ~~In none of the three colonies of the test substance variant an abnormal decrease of the colonies strength and the bee brood development was observed.~~ The continued presence of eggs showed that queens were in good condition in all colonies of the test substance variant. There was a decline in capped brood, and low number of larval stages in both the treatment and control groups. In the treatment group, the number of larvae stages on DAE 9/DAE 28 for each colony were 1.7/0 (colony 1), 0/0 (colony 2) and 0/5 (colony 3), compared to the control on DAE 9/DAE 28 for each colony which were 0/0 (colony 1), 0/0 (colony 2) and 10/10 (colony 3). **Conclusion:** ~~According to the results it is concluded that the dressing of oil seed rape with A 9700 B did not cause an intoxication of adult honey bees. Furthermore there were no effects on the strengths of the colonies, egg laying rate of the queen and the bee brood development~~. According to the results it is concluded that there were similar observations of mortality, and decline in brood in both the treatment variant and the control variant.
Uncertainties and notes	• It is unclear if there were enough sampling intervals for brood and colony condition to show development in the hives. • There is uncertainty surrounding the drilling practice compared to current drilling practice in Canada. • For mortality data, in table 8, DAE mortality in the control variant in colony 2 was extremely high (148 and 95 dead bees in the bee trap and edge of crop assessments, respectively). These were removed from analysis by the study authors because they were considered outliers (which the PMRA reviewer agrees with) – however, the average mortality (calculated as 6.7 by the study author) was the sum divided by 3 colonies. It should have been divided by 2 colonies since one was removed. The average should be 10 dead bees, not 6.7. This makes the difference between the treatment group and control group mortality less notable. • Note: The mortality in both the treatment and control variants were much higher in the edge of crop assessment. [The PMRA reviewer questions if this observation is due to bees attempting to forage beyond the tent for additional nutrition (?)]. However, overall, the number of dead bees was not high (< 20 in control and trt). • Residue analysis should have been conducted on various matrices e.g., bee pollen and nectar, comb pollen and nectar to confirm the level of exposure of thiamethoxam to the bees. • The data for bee mortality, flight intensity and brood development should have been presented in graphs with a measure of variability (since there were 3 treatment replicates) to allow for an easier interpretation. • Statistics (e.g., paired t-test) should have been performed on the data.

	• Mortality assessments were only made for 8 days. • Stored food was not removed prior to exposure. It is unknown if brood would have had sufficient exposure. • The study did not include a toxic reference.
Criteria	• The following criteria for the colonies were guaranteed: • at least 2 brood combs containing eggs, larvae and capped cells • at least 1 honey and pollen comb • bees are free of symptoms of Nosema and other bee diseases (veterinary • certificate of good health)
NOTE	An open container with water was placed into each tunnel. The surface of the water was covered with e.g. scraps of polystyrene to prevent the bees from drowning.

Summary Tables of results	**see** [HYPERLINK \l "_APPENDIX_1-_SUMMARY"]

EAD Evaluator comments (including acceptability and its use in the risk assessment):

EAD NOTES: This study is considered 'informative' for PMRA and the information will be used in a line of evidence approach in the risk assessment. Uncertainties and limitations will be outlined. The residue data can be used by both agencies in the overall risk assessment.

A9700B is Cruiser 350 FS, which is a relevant EUP for Canada, registered for Wheat, barley, corn, rye, triticale, buckwheat, millet, sorghum, soybeans and beans at 62.5 g ai/ha maximum rate, or 0.25 mg per kernel for corn (up to 100 g ai/100 kg seed).

EAD summary (for monograph): The purpose of this semi-field study was to examine the effects of oil-seed rape treated seed on honey bees. Plots (4.8 m x 3.6 m x 2 m height) were planted with spring oil-seed rape (*Brassica napus*) dressed with A-9700 B (at 1200 mL thiamethoxam/100 kg seed), and then hives (3 replicates) were introduced at flowering for 8 days. There was also a concurrent water control.

Mortality and foraging activity were assessed before and during the exposure period until day 8. Condition of the colonies and the development of the bee were assessed before exposure, on day 9 (just after exposure) and also on day 28.

Mortality:
The mortality in the test substance variant was in the same range as mortality of the control variant. The average mortality during the evaluation days day 1to 8 was 14.1 dead bees/colony and day in the treated variant and 17.8 dead bees/colony and day in the control variant. Based on mortality on individual days of observation, there was slightly higher mortality on the first day after introduction in the treatment group (17.7 dead bees) compared to the control (10 dead bees). Thereafter, the control mortality and treatment mortality showed a similar trend. A higher number of dead bees were found in the edge of crop assessment (compared to the bee trap assessment) in the last few days of the experiment, in both the treatment and control groups.

Foraging activity:
Regarding the flight intensity only negligible differences were observed between the test substance and control variant. During the entire observation period the average flight intensity was 7.3 bees/metre squared in the three replications of the test substance variant compared to 7.6 bees/metre squared in the control variant.

Brood development:
The continued presence of eggs showed that queens were in good condition in all colonies of the test substance variant. There was a decline in capped brood, and low number of larval stages in both the treatment and control groups. In the treatment group, the number of larvae stages on DAE 9/DAE 28 for each colony were 1.7/0 (colony 1), 0/0 (colony 2) and 0/5 (colony 3), compared to the control on DAE 9/DAE 28 for each colony which were 0/0 (colony 1), 0/0 (colony 2) and 10/10 (colony 3).

Conclusion: According to the results it is concluded that there were similar observations of mortality, and decline in brood in both the treatment variant and the control variant.

EAD Primary Evaluator (Officer No.): 1183
Date: September 4th 2014

Foreign review comments, if available (state agency): Notes from EFSA 20 Dec 2012

Evaluation		
	Positive evidence for exposure	Negative evidence for exposure
Exposure	• Flight intensity assessments[3] confirmed foraging on the treated plots. Flight intensity in the treated tunnels (mean 7.3 bees per m^2 per day) was comparable to the control plots (mean 8.1 bees per m^2 per day). • The study design was a tunnel study and therefore the bees did not have an alternative foraging area during the exposure period.	• Mortality assessments were only made for 8 days. • Final brood assessments were made on 28 DAEs. • Stored food was not removed prior to exposure. • The study design was a tunnel study and therefore there is only limited area for the bees to forage. The effects if bees had to travel over further distances to obtained food (i.e. use more energy) is not covered. • No toxic reference was used. • Only three assessments of bee brood were made. • No residue analysis was performed.

Exposure to forager bees	Forager bees were observed to forage on the treated crop during the study. Given that it was a tunnel study the bees had no alternative to forage elsewhere. However, as no residue analysis was performed it is not possible to confirm the level of exposure.
Exposure to adult in-hive bees	Forager bees were observed on the treated crop and therefore are likely to have brought pollen and nectar from the treated crop back to the hive. However, as food stocks were not removed prior to study initiation and the study was of limited length and no residue analysis was performed, it is not known whether there was exposure to in-nest bees.
Exposure to brood	Forager bees were observed on the treated crop and therefore are likely to have brought pollen and nectar from the treated crop back to the hive. However, as food stocks were not removed prior to study initiation and the study was of limited length and no residue analysis was performed, it is not known whether there was exposure to bee brood.
General (including positive aspects of note)	The study to GLP and was well performed. The study was performed in accordance with EPPO 170.
Limitations	• Mortality assessments were only made for 8 days. • No long-term assessments or overwintering assessments made. • No assessments were made for bee trap mortality before and after the exposure period. Background mortality for the colonies used in the study (i.e. similar to an internal control) was therefore unknown. • No toxic reference was used which is recommended in EPPO 170. It is acknowledged that there is no agreed toxic standard for seed treatments. • Bee behaviour assessments were included; however, it was not clear that the assessment was done in a systematic way (i.e. detailed assessments). Only some sublethal effects were included in assessment. • Plot size was 3.6 x 2.4 m (8.64 m^2) which is less than recommended in EPPO 170. • Only three brood assessments were made. • Food stocks were not removed prior to the exposure period.
Forager bee mortality	The mean daily mortality in the treatment colonies was 14.1 dead bees/day (Table 7 of the study report). The mean daily mortality in the untreated control colonies was 17.8 dead bees/day (value excludes the mortality observed in C2 1 DAE as the study author assessed these to be outliers) (Table 8 of the study report).
In-nest adult bee mortality	No specific assessment of in-nest bee mortality was performed.
Bee behaviour	The study author mentioned that no abnormal bee behaviour was observed. However, no systematic results were included in the study report.
Bee brood	Three brood assessments were made. The study author noted that there was a decline of bee brood n the treated colonies and the control colonies. The study author proposed that this was due to the study design. The study author noted that the continuous presence of eggs indicated that the queen was in good health. However, due to the study design it is considered unlikely that there was sufficient opportunity for bee brood to be fed with contaminated food.
Overall	Although the study was well performed, due to age and design of the study, it is considered to provide limited information for risk assessment.

[1] Flight intensity assessments are presented in tables 19 to 20 and summarised in figures 2 of the study report.

Sub. No.: 2012-1919 EAD DER No.

EAD peer review comments (agree/disagree/issues): Residue analysis should have been conducted on various matrices e.g., bee pollen and nectar, comb pollen and nectar to confirm the level of exposure of thiamethoxam to the bees. The data for bee mortality, flight intensity and brood development should have been presented in graphs with a measure of variability (since there were 3 treatment replicates) to allow for an easier interpretation. Statistics (e.g., paired t-test) should have been performed on the data.

EAD Secondary Reviewer (Officer No.): 216
Date: January 14, 2015.

Any additional registrant comments, if applicable:

APPENDIX 1- SUMMARY TABLES

Tab. 1: Details about sowing.

Date		04JUN1998
Time		10.45 a.m. - 11.30 a.m.
Seeding machine		Pneumatic seeder : Accord Pneumatic I A
Sowing rate	[kg/ha]	14.5
Sowing depth	[cm]	2 - 3
Soil temperature	[° C]	16

Tab. 7: Individual results of the evaluations of mortality (numbers of dead bees) in the A-9700 B variant.

Date	DAE	Mortality (number of dead bees)						
		Colony 1		Colony 2		Colony 3		∅/Colony and day
		BT	E	BT	E	BT	E	
04AUG98	1	12	9	5	11	10	6	17.7
05AUG98	2	1	6	0	1	2	2	4.0
06AUG98	3	3	7	1	3	1	3	6.0
07AUG98	4	2	7	2	8	4	4	9.0
08AUG98	5	0	14	0	8	4	9	11.7
09AUG98	6	0	38	1	24	1	8	24.0
10AUG98	7	3	29	1	19	3	17	24.0
11AUG98	8	1	22	0	11	1	15	16.7
Mean		2.8	16.5	1.3	10.6	3.3	8.0	14.1
STD		3.9	12.0	1.7	7.7	3.0	5.5	7.7

DAE = Days after exposure
BT = Bee traps
E = Edge of the crop
STD = Standard deviation

Tab. 8: Individual results of the evaluations of mortality (numbers of dead bees) in the control variant.

Date	DAE	Mortality (number of dead bees)						
		Colony 1		Colony 2		Colony 3		∅/Colony and day
		BT	E	BT	E	BT	E	
04AUG98	1	4	7	148*	95*	2	7	6.7
05AUG98	2	3	0	3	8	2	3	6.3
06AUG98	3	4	1	4	25	3	12	16.3
07AUG98	4	2	3	7	35	1	7	18.3
08AUG98	5	2	12	0	39	2	24	26.3
09AUG98	6	0	5	1	44	4	18	24.0
10AUG98	7	0	8	1	62	3	17	30.3
11AUG98	8	4	0	0	27	0	10	13.7
Mean		2.4	4.5	2.3	34.3	2.1	12.3	17.8
STD		1.7	4.3	2.6	16.9	1.2	7.0	8.8

DAE = Days after exposure
BT = Bee traps
E = Edge of the crop
STD = Standard deviation
* = values identified as outliers according to Dixon (1953)

Tab. 9: Average flight intensity (number of bees per m² *Brassica napus*) in the three colonies of the A-9700 B variant.

Date	DAE	Flight intensity (number of bees per m²)			
		Colony 1	Colony 2	Colony 3	∅/Colony and day
04AUG98	1	4.7	5.2	4.7	4.9
05AUG98	2	10.0	8.0	8.9	9.0
06AUG98	3	10.1	8.8	9.9	9.6
07AUG98	4	9.3	8.0	12.3	9.9
08AUG98	5	13.0	15.0	15.0	14.3
09AUG98	6	5.0	8.0	8.0	7.0
10AUG98	7	1.0	1.0	4.3	1.3
11AUG98	8	0.7	2.0	3.0	1.9
Mean		6.7	7.0	8.3	7.3
STD		4.6	4.4	4.2	4.3

DAE = Days after exposure
STD = Standard deviation

Tab. 10: Average flight intensity (number of bees per m² *Brassica napus*) in the three colonies of the control variant.

Date	DAE	Flight intenstity (number of bees per m²)			
		Colony 1	Colony 2	Colony 3	∅/Colony and day
04AUG98	1	7.8	4.9	5.6	6.1
05AUG98	2	10.0	10.1	11.2	10.4
06AUG98	3	8.7	8.3	9.9	12.6
07AUG98	4	9.0	9.3	12.0	10.1
08AUG98	5	12.0	14.0	16.0	14.0
09AUG98	6	5.0	7.0	9.0	7.0
10AUG98	7	0.7	1.0	4.3	2.0
11AUG98	8	2.3	0.7	4.3	2.4
Mean		6.9	6.9	9.0	8.1
STD		3.9	4.6	4.1	4.5

DAE = Days after exposure
STD = Standard deviation

Tab. 11: Brood development of the A-9700 B variant.

	Colony 1	Colony 2	Colony 3
Prior to exposure of the bees: 03AUG98 (DAE -1)			
Strength (No. of combs covered with bees)	3	3	3
Average amount of pollen and nectar in %	43.4	36.7	53.3
No. of combs covered with brood	3	2	3
Average amount of egg stage in %	11.7	30.0	13.3
Average amount of larval stage in %	1.7	2.5	1.7
Average amount of capped stage in %	23.3	27.5	18.3
1st assessment after exposure: 12AUG98 (DAE 9)			
Strength (No. of combs covered with bees)	3	3	3
Average amount of pollen and nectar in %	46.7	26.7	51.7
No. of combs covered with brood	3	2	3
Average amount of egg stage in %	5.0	15.0	13.3
Average amount of larval stage in %	1.7	0	0
Average amount of capped stage in %	11.7	10.0	6.7
2nd assessment after exposure: 31AUG98 (DAE 28)			
Strength (No. of combs covered with bees)	3	3	3
Average amount of pollen and nectar in %	33.3	43.3	56.7
No. of combs covered with brood	1	1	1
Average amount of egg stage in %	20.0	5.0	10.0
Average amount of larval stage in %	0	0	5.0
Average amount of capped stage in %	10.0	0	0

Tab. 12: Brood development of the control variant.

	Colony 1	Colony 2	Colony 3
Prior to exposure of the bees: 03AUG98 (DAE -1)			
Strength (No. of combs covered with bees)	3	3	3
Average amount of pollen and nectar in %	46.7	46.7	60.0
No. of combs covered with brood	2	2	1
Average amount of egg stage in %	20.0	10.0	20.0
Average amount of larval stage in %	0	10.0	0
Average amount of capped stage in %	25.0	35.0	40.0
1st assessment after exposure: 12AUG98 (DAE 9)			
Strength (No. of combs covered with bees)	3	3	3
Average amount of pollen and nectar in %	53.3	33.3	63.3
No. of combs covered with brood	2	1	1
Average amount of egg stage in %	20.0	10.0	20.0
Average amount of larval stage in %	0	0	10.0
Average amount of capped stage in %	5.0	40.0	10.0
2nd assessment after exposure: 31AUG98 (DAE 28)			
Strength (No. of combs covered with bees)	3	3	3
Average amount of pollen and nectar in %	51.7	30.0	53.3
No. of combs covered with brood	0	1	1
Average amount of egg stage in %	0	30.0	10.0
Average amount of larval stage in %	0	0	10.0
Average amount of capped stage in %	0	10.0	10.0